Inflammatory and Cardiovascular Responses to Inhaled Fine Particles

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Introduction

. Recent epidemiological and toxicological studies have associated human morbidity and mortality, injury to the lung and changes in cardiac functions with episodes of elevated PM concentrations.

. Individuals with pre-existing lung and heart diseases are among the most sensitive individuals.

. There is evidence that PM exposures are associated with increased risk of cardiovascular diseases, including myocardial infarctions (heart attacks), hypertension and decreases in heart rate variability.

Hypotheses

- The primary objectives of this project are:
  - to examine the cardiopulmonary health effects of atmospheric mixtures that realistically model sizes and compositions of particles in California air;
  - to examine mechanisms that mediate systemic changes and other adverse effects of inhaled particles.
- A secondary objective was to examine the interaction of ozone and PM.
- We tested the following hypotheses:
  - Inhaled fine particles would cause inflammation in the lung and release mediators that could alter blood pressure and heart rate;
  - Multiple day exposures would elicit more severe cardiopulmonary responses than would single day exposures.

Approach

- This project was a component of a larger multicampus, interdisciplinary research program.
- The studies performed at UCI complemented the human clinical studies of air pollution effects at UCSF.
- The studies at UCI focused on fine particles and their role in inducing cardiopulmonary responses in a sensitive animal model, the senescent or ‘geriatric’ rat.

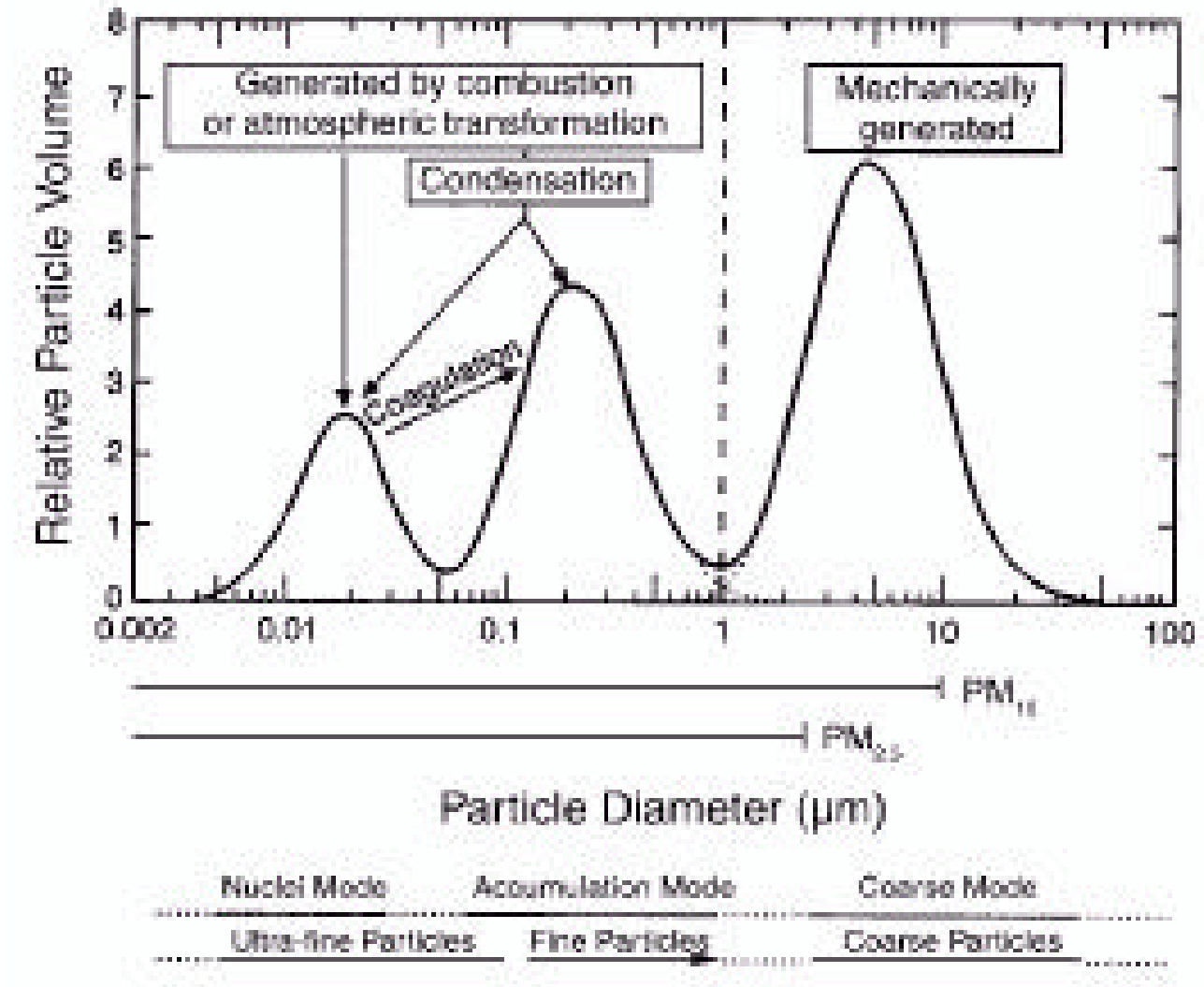
Specific Aims

- Determine the cardiopulmonary responses to combination ozone-particle inhalation in a sensitive laboratory animal model.
- Compare the effects of single day PM exposure with those of multiple-day exposures to PM.
- Determine the effect of the total dose of inhaled particles on airway inflammation, cellular function, and cardiovascular responses as compared with immediate responses.

Overall Integration

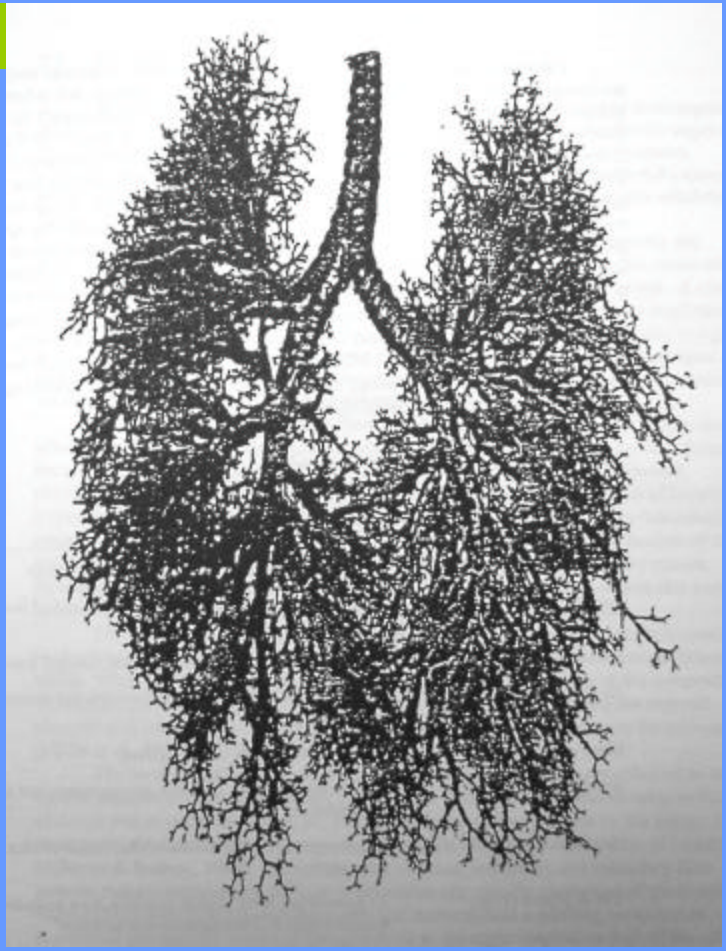
- To maximize the comparability of the studies in human and animal models,
  - Exposures were conducted for 4 hours per day for one day and three days.
  - Particles were composed of ammonium nitrate (AMN; 150 $\mu g/m^3$) and elemental carbon (EC; 100 $\mu g/m^3$).
  - Ozone concentrations were 0.2 ppm.
  - Particle sizes were ~0.7µm mass median diameter.

Three Size Modes of Particles in Ambient Air Originate from Different Processes.



Understanding Particle-Induced Health Effects Begins With Understanding the Lung

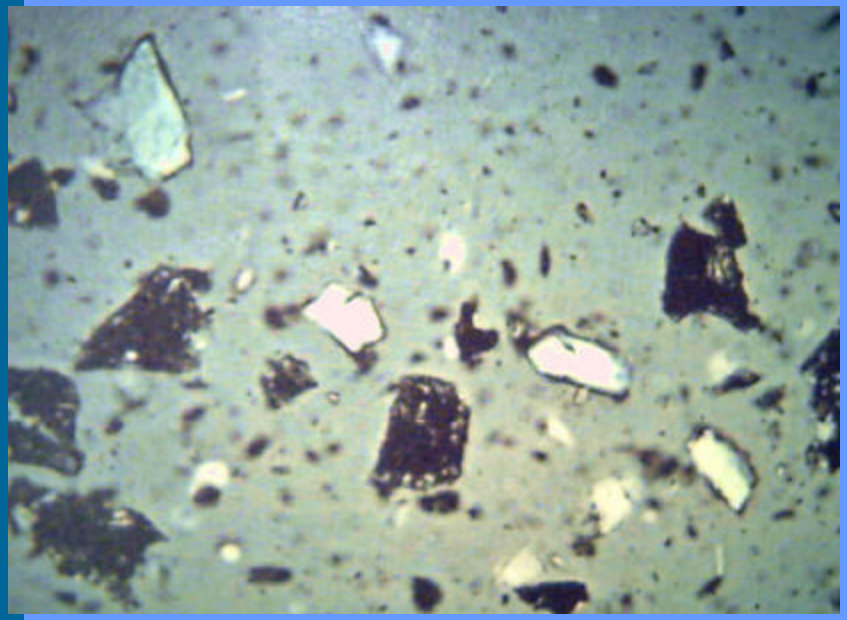
- The human lung is a complex, branching structure.
- The structure is also complex at the cellular level .
- This complexity results in differential sensitivity to particles.

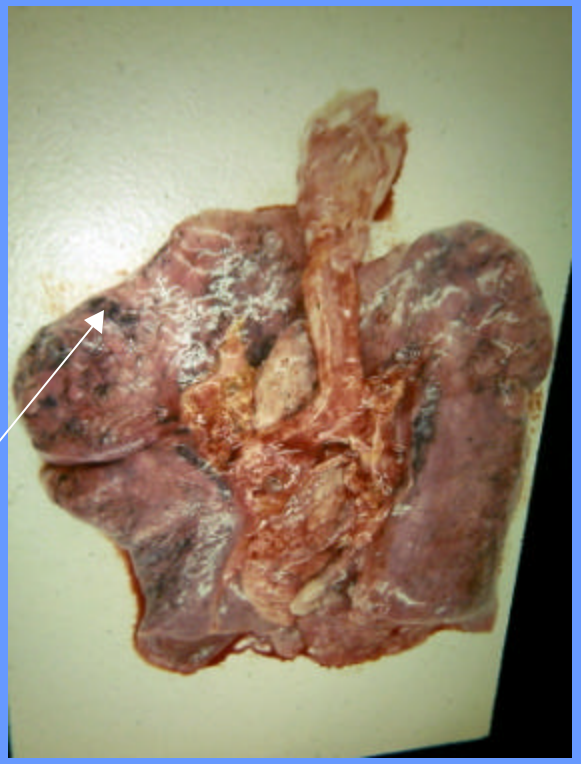


Acute Respiratory Tract Injury

- Conducting airways and gas exchange region of the lung can be injured by inhaled particles.
  - At rest, 7-10 LPM ventilation.
  - Increased by exercise.
  - Lung capillary bed perfused 1 to 5 times by ENTIRE circulating blood volume.
- Injury patterns are different for oxidant gases (e.g. O_3) and particles.
  - O_3 - centriacinar focal lesions.
  - Particles – Diffuse lesions and interstitial inflammation.

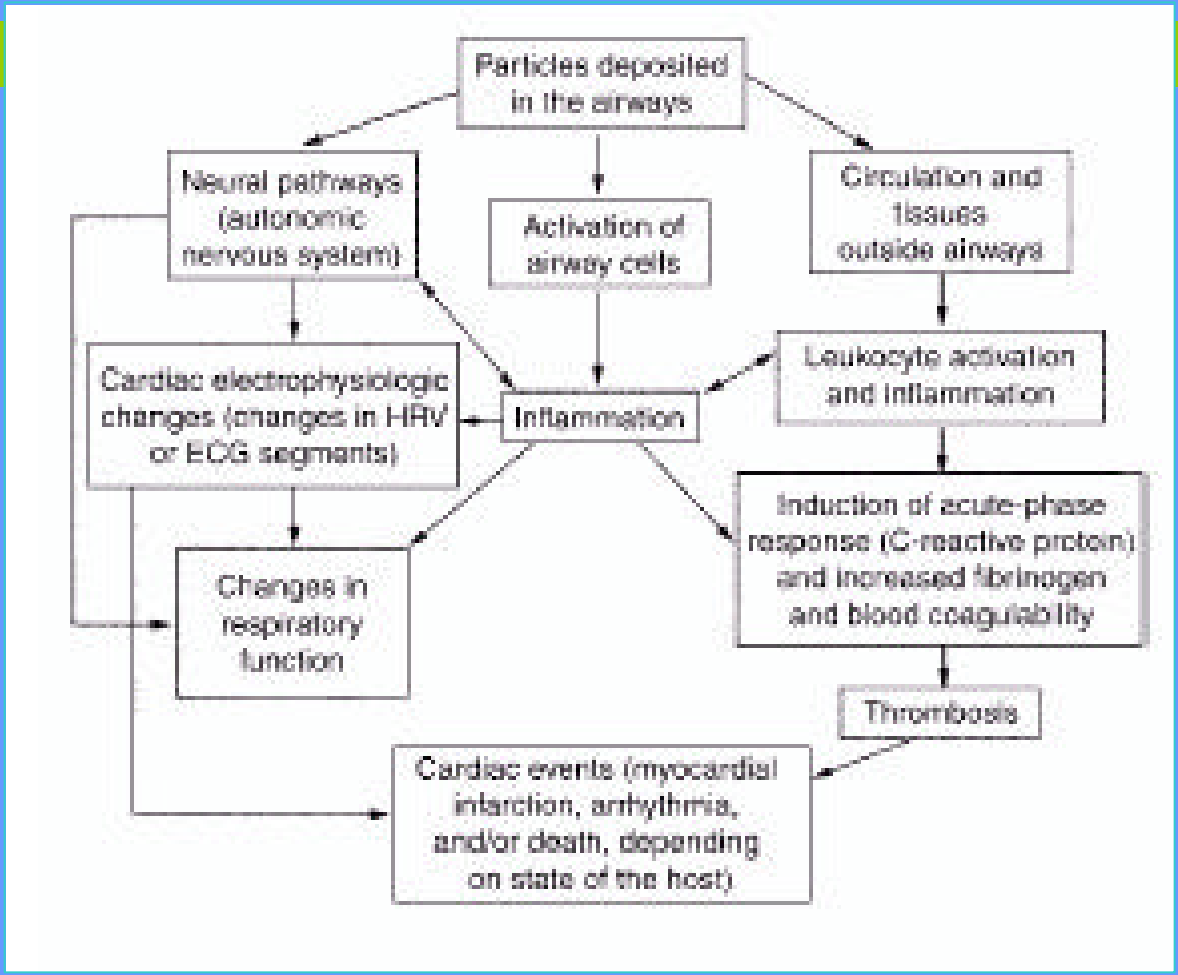
Ambient Particles Are A Complex Mixture of Soluble and Insoluble Components.



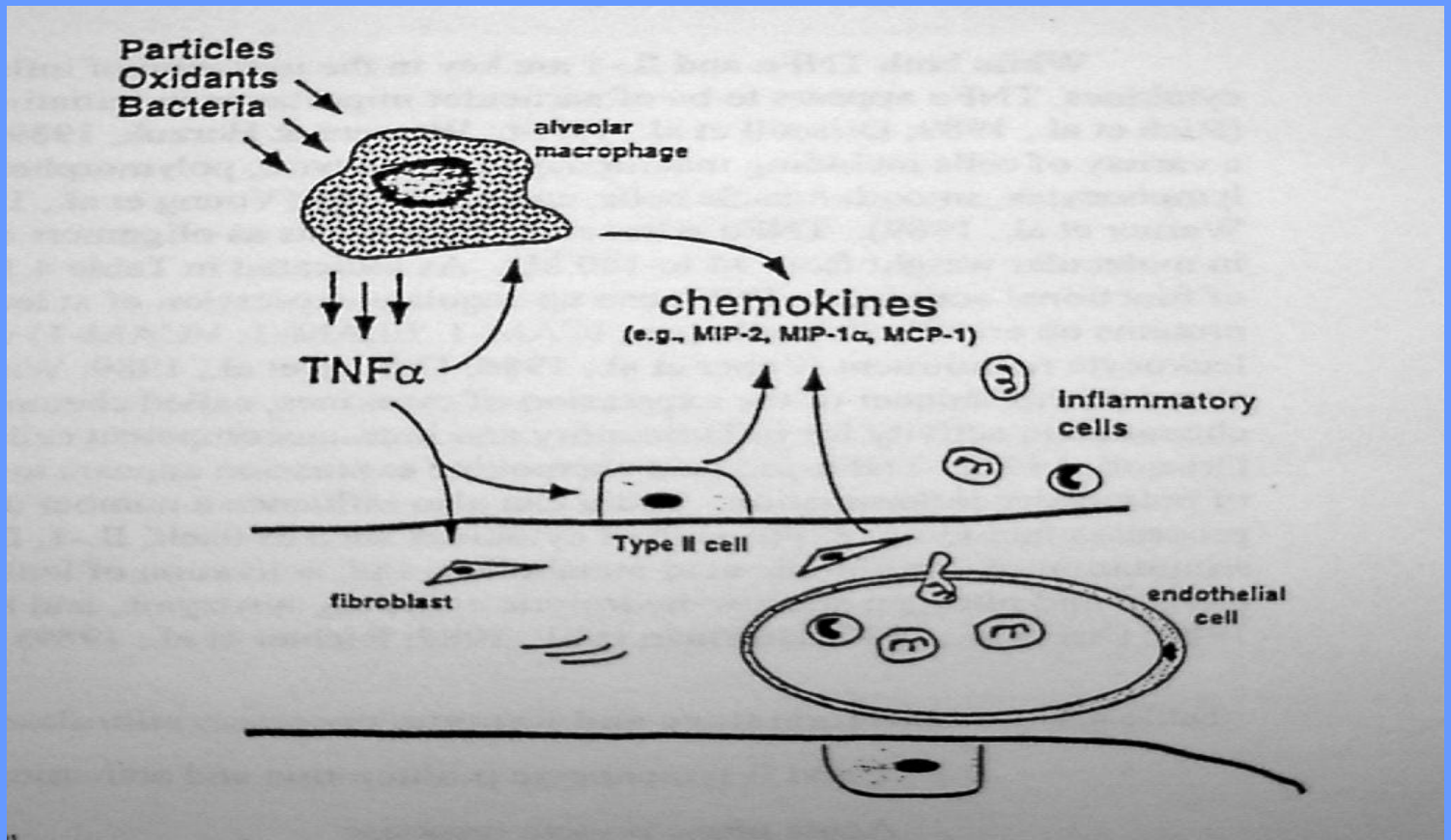


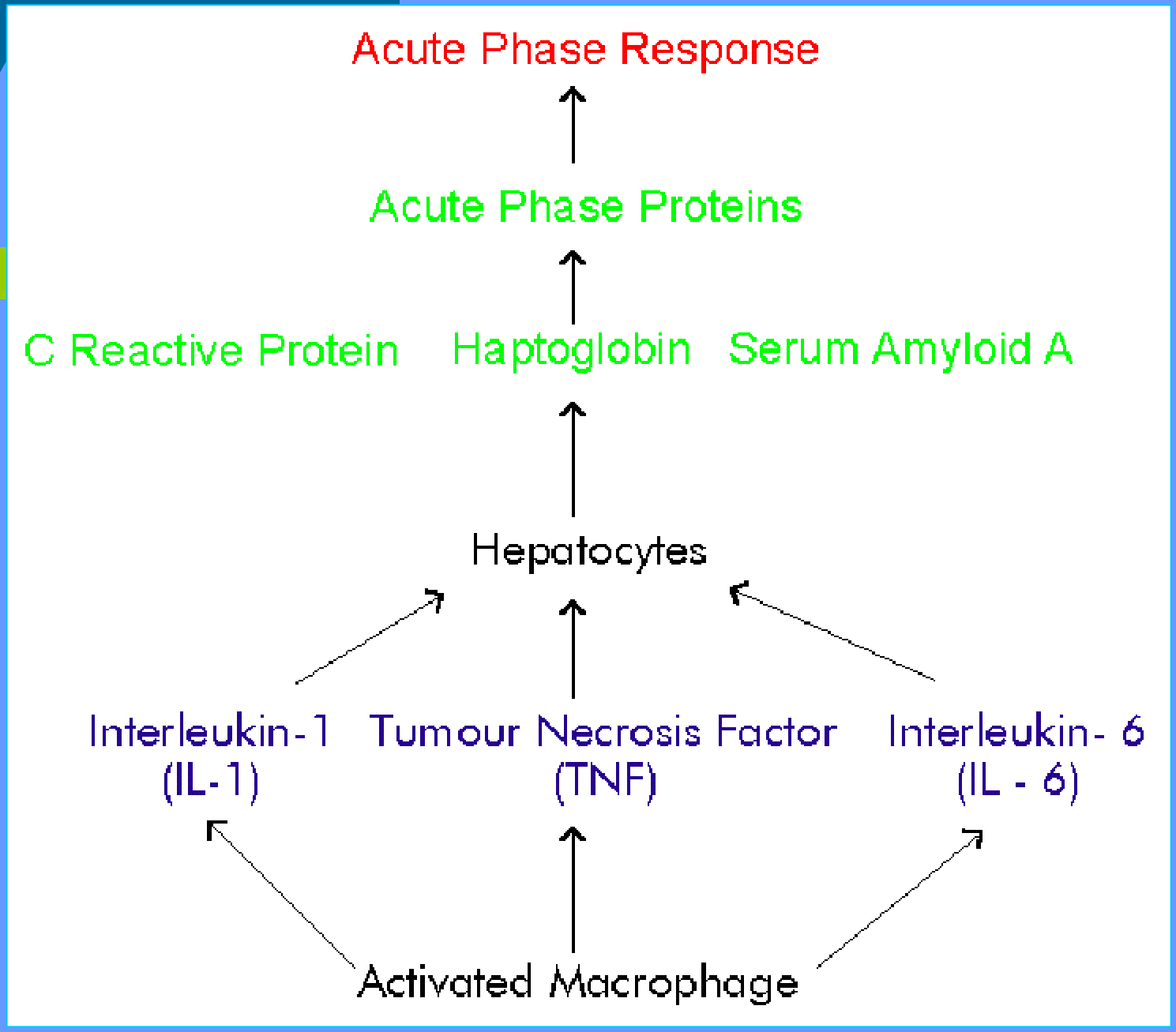
Insoluble components can accumulate over time leading to chronic inflammation and disease

Particle deposition in airways can activate the innate immune system and induce adverse effects in airways and the cardiovascular system.



Cytokines and Chemokines elicited by cells of the innate immune system can enter systemic circulation




Acute Phase Response
Acute Phase Proteins
C Reactive Protein
Haptoglobin
Serum Amyloid A
Hepatocytes
Interleukin-1 (IL-1)
Tumour Necrosis Factor (TNF)
Interleukin- 6 (IL - 6)
Activated Macrophage

Measures of Lung Injury

- Cell Proliferation
  - Mechanism for replacement of injured epithelial cells (injury repair)*.
  - Interstitial proliferation may indicate both repair and ‘recruitment’ of inflammatory cells*.
  - Sustained cell proliferation in a tissue may enhance tumor development (review of available evidence is equivocal).

Measures of Lung Injury (Cont.)

- Edema
  - Influx of plasma and cells to interstitial and airway-alveolar airspaces.
  - May be associated with alterations of microvascular and epithelial function (e.g. opening of tight junctions).
  - Runs gamut from minor or quickly reversible ultrastructural changes to disruption of entire cell layer (rapidly fatal).
  - Often accompanied by oxidative stress.
  - Consequences can be gas-exchange compromise and resolution can involve fibrogenesis leading to permanent or progressive damage.

Measures of Lung Injury (Cont.)

- Oxidative Stress
  - Free radicals in lung*
  - NO
  - Can be produced by inhaled particles (transition metals) or by phagocytic cells secondary to ingesting particles (respiratory burst).
  - Decreased concentrations of anti-oxidants.
  - Inactivation of anti-proteases.
- Inflammatory Mediators
  - Arachidonic Acid Metabolites
    - Prostaglandins
    - Leukotrienes
  - Complement system

Measures of Lung Injury (Cont.)

- Cytokines
  - Small glycosylated proteins (8 to 30 kDa)
  - Regulate cell differentiation, proliferation and activation
  - Interact with specific cell membrane receptors on immune and non-immune system cells.
  - Elaborated by cells to regulate their own physiology (autocrine) or that of other cells (paracrine).
  - Influence physiological processes (e.g. body temperature, apetite, sleep patterns).
  - Role(s) in tissue injury, infection and neoplasia are complex.
  - Can be “initiators” or “recruiters.”

Measures of Lung Injury (Cont.)

- Initiating Cytokines (IL-1, TNFα, TGFβ)
  - Early mediators of response.
  - Expressed rapidly after exposure to toxic agent or recognition by host defenses of infection or neoplasia.
  - Can set ‘cascades’ in motion and can induce cytokine expression and responses in non-immune cells (fibroblast, smooth muscle, epithelial and endothelial cells).
  - Persistent expression of initiating cytokines can be associated with chronic inflammatory or fibrotic lung diseases (e.g. pulmonary granulomas and tissue fibroses).

Measures of Lung Injury (Cont.)

- Cytokines recruit inflammatory cells.
- Cytokines elicit changes that allow recruited cells to “acquire” targets.
  - Elicit adhesion molecules.
  - Stimulate proliferation of epithelial cells.

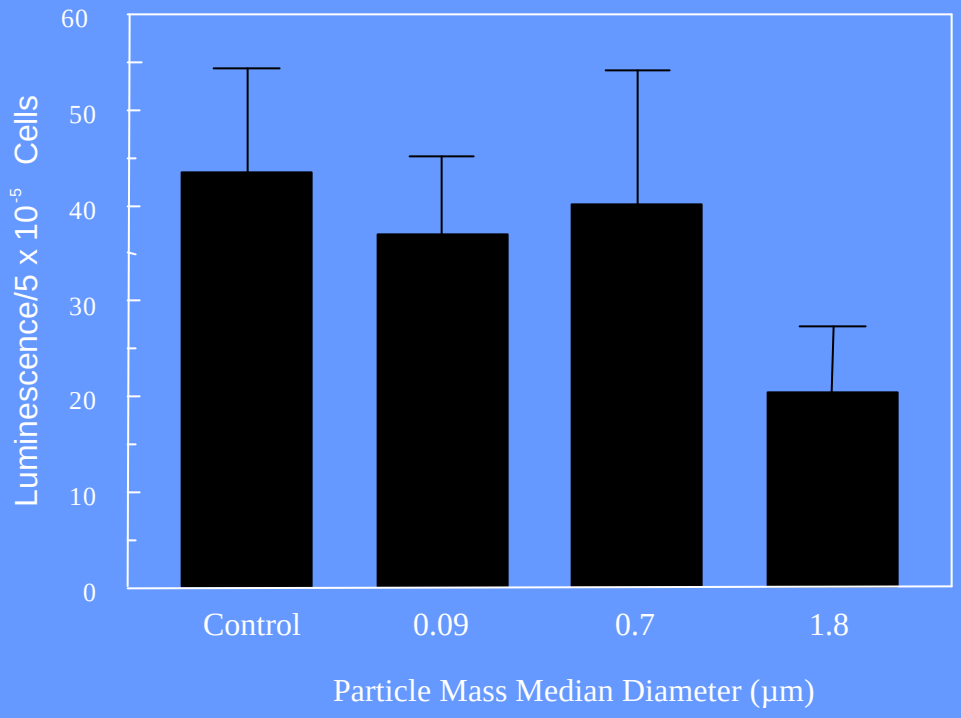
Results that Will Be Discussed

- Initial study that showed although particle size was important, DOSE was most likely the critical factor, and that inflammation and oxidative stress were associated with cardiovascular changes.
- This study that showed that ozone effects were important in single day exposures, but particles could have an independent effect in 3-day episodes.
- A pilot study that showed that rats exposed to ambient particles behaved similarly to rats exposed to laboratory-generated particles.

Exposure Data for the Particle Size Study

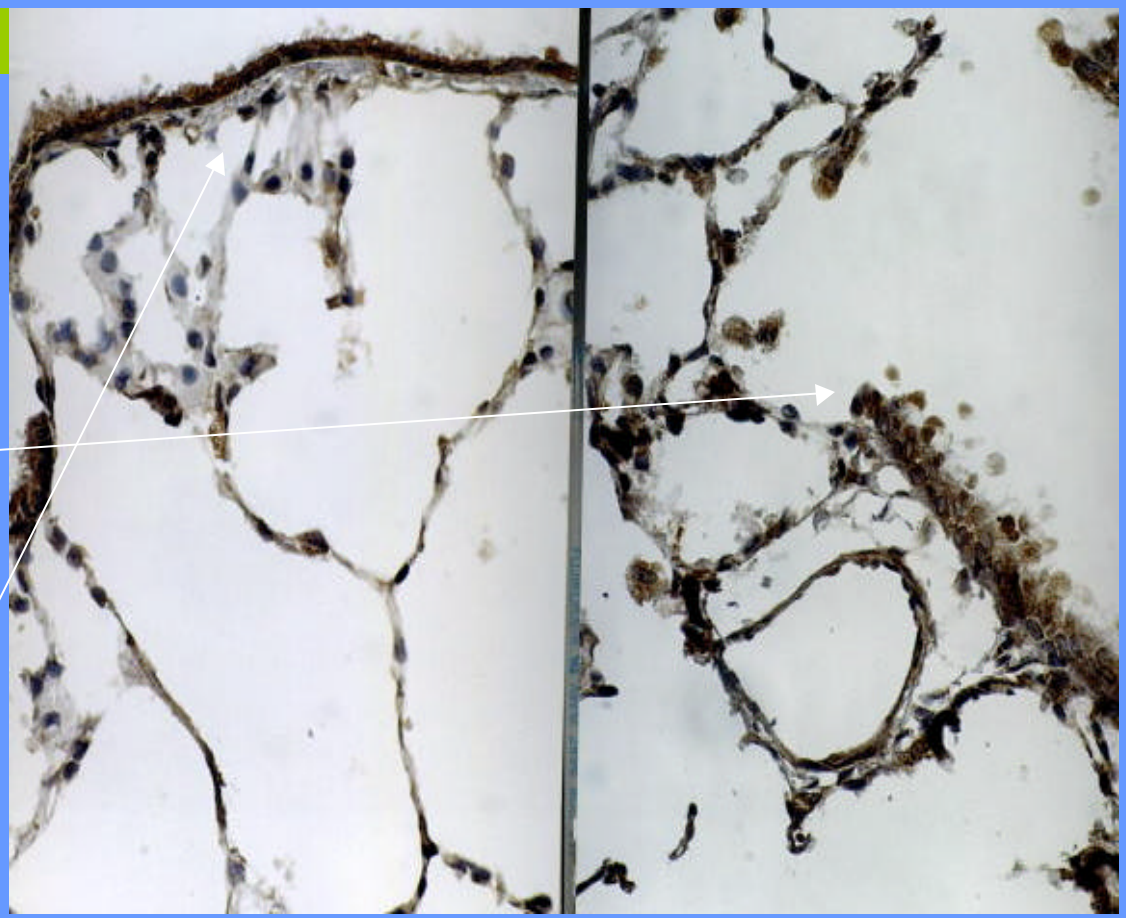
Components	AMN	300 μg/m^3
	C	200 μg/m^3
	O_3	0.2 ppm
Large	1.2 μm MMAD	3.2 GSD
Fine	0.6 μm MMAD	2.8 GSD
Ultrafine	0.09 CMD	

Respiratory Burst Activity In Macrophages from Aged Rats

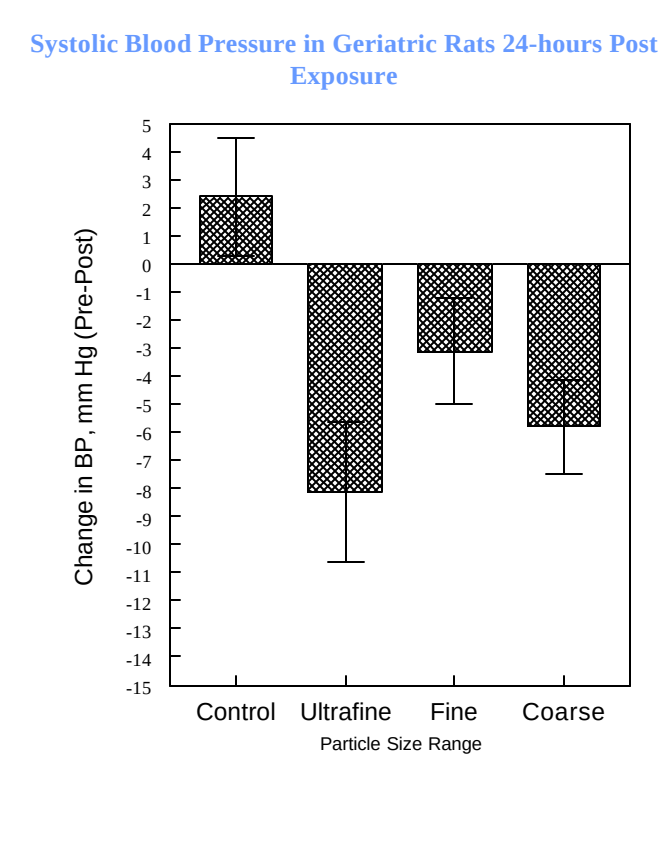


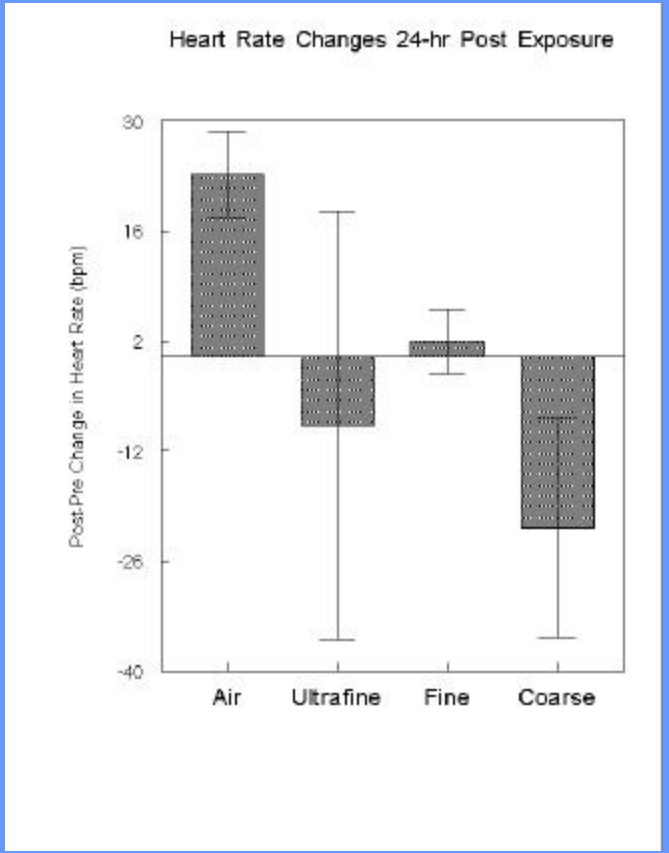
Evidence of Oxidative Stress in Lungs of Rats

- Terminal bronchioles stained for nitrotyrosine
- Note intense staining associated with inflammatory cells in Panel B.
- Note unstained alveolar epithelium in control (Panel A).



Hemodynamic Changes After Particle Exposures in Geriatric Rats



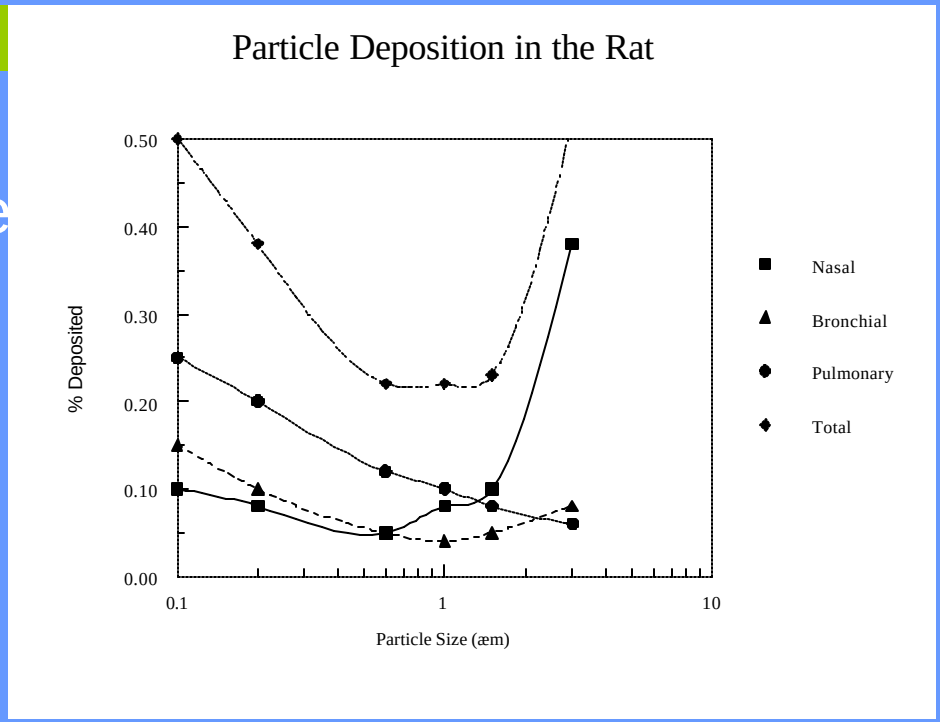


It is important that we examine these data in terms of dose!

- For within group comparisons
- For extrapolation purposes!
- This is done using deposition models and knowledge of respiratory parameters and particle size distributions.

Particle Size Determines Deposition Fraction and Dose

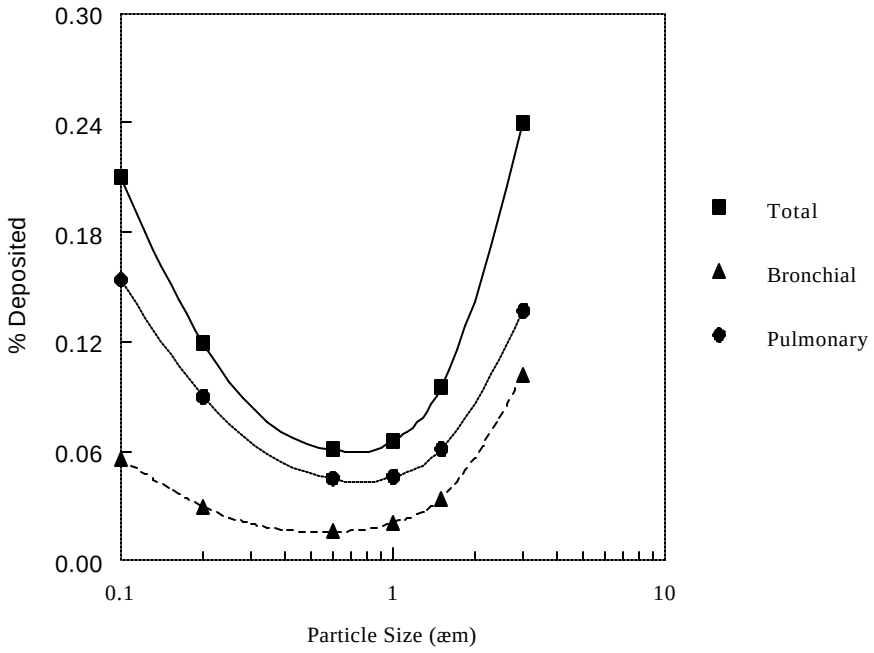
- Nasal deposition is markedly greater for the Coarse Particles.
- Deposition in the gas exchange region of the lung increases with decreasing particle size.



Particle Size Determines Deposition Fractions and Sites

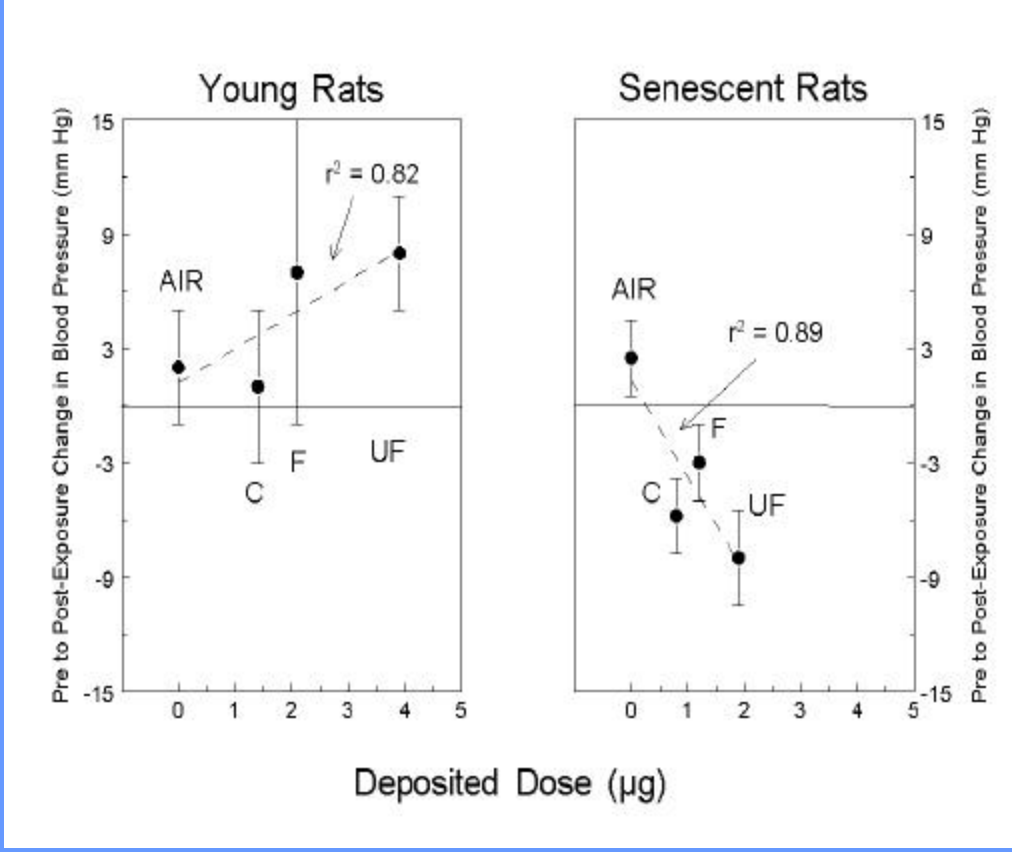
- Thoracic deposition can be estimated using appropriate models after correcting for nasal deposition.
- Of the aerosol that penetrates through the URT, most deposit in the gas exchange region.

Figure 2. Estimated Regional Particle Deposition in the Rat



Particle Mass Deposition and Dose Significantly Relate to Blood Pressure Response

- We can estimate deposition fractions using models.
- We can estimate ventilation from observations or scaling models.
- We can calculate a deposited dose.



This Experiment

- Rats were exposed to laboratory-generated particles (EC + AMN) at 250 µg/m^3 4 hr per day for 1 day and 3 days.
- Only one particle size was used (0.7 µm)
- BP, HR and HRV were evaluated

Methods (Exposure)

- Rats were exposed nose-only for 4 hr.
- Atmospheres contained ammonium nitrate (AMN), elemental carbon (C) and ozone (O_3).
- Rats were exposed to filtered air (FA), particles alone (PM) or to a mixture of particles and ozone (PO).

Methods (Endpoints)

- Blood pressure and ECG measured immediately before exposure and after exposure.
- Rats were euthanized 18-hr post-exposure.
- Left lung was lavaged; right lung was inflated and fixed for histology.
- BAL was tested for inflammatory cell infiltration.

Single Day Exposures

Inflammatory Cells in BAL and Cardiovascular Responses of Rats Exposed to Ammonium Nitrate and Carbon Particles (PM) in the Presence or Absence of Ozone (O3), N = 10 per group.

(Number of Cells; Mean SE)

Atmosphere	Total Cell Number (x 10^{-6})	Macrophages (x 10^{-6})	PMNs	Lymphocytes
FA	3.8 0.3	3.7 0.3	26600 2400	95000 19000
PM	3.7 0.2	3.6 0.2	29600 3500	67000 23000
PO	3.4 0.3	3.3 0.3	70400 5400*	37400 5400

Note: *p= 0.05

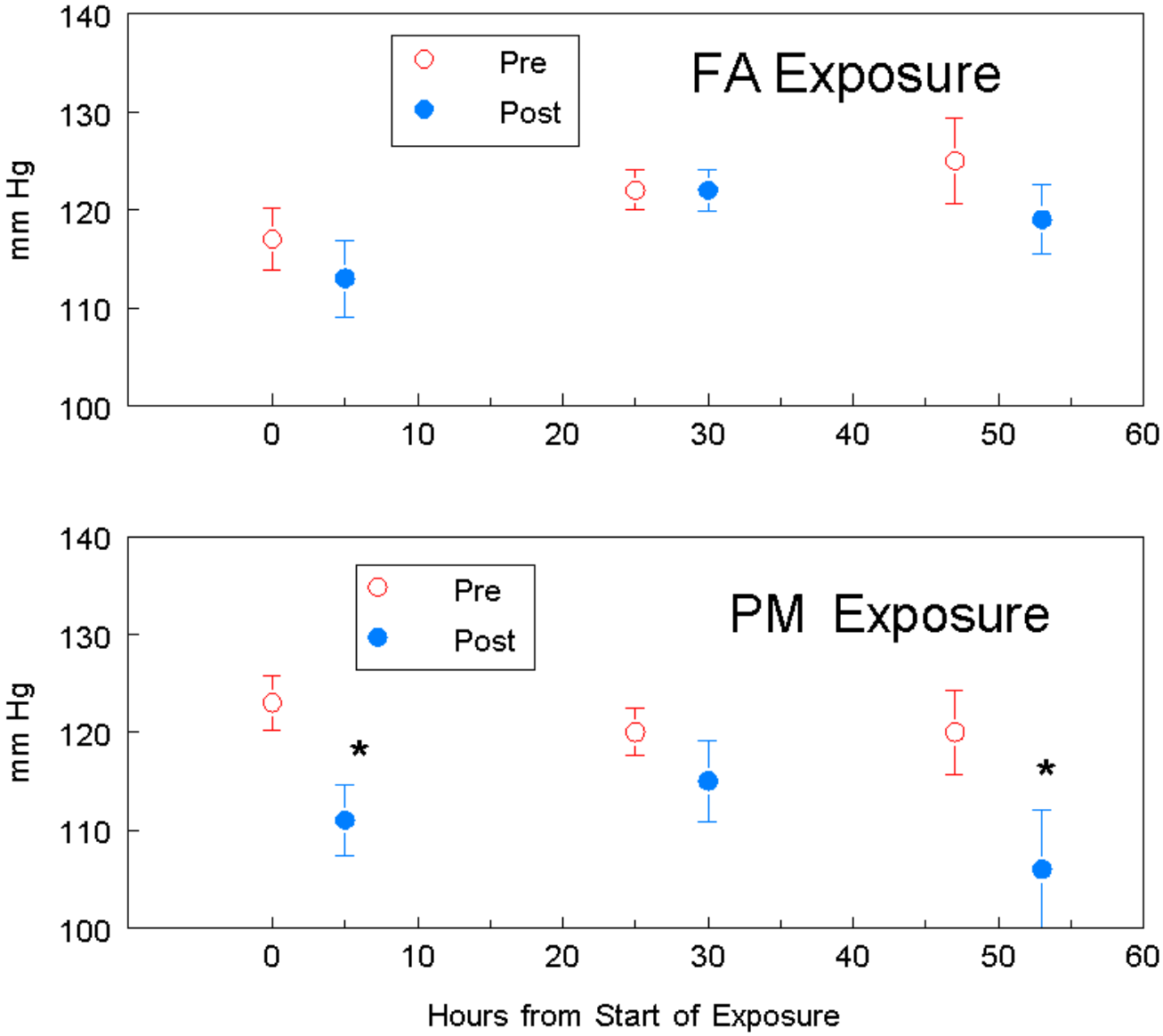
Exposure	SBP[a]	HR[b]	DP[c]
FA[d] (n=6)	99 4	422 17	42000 1800
PO[e] (n=6)	103 4	420 17	43000 2300
PM[f] (n=10)	110 4	402 7	45000 1900

Notes: [a]Systolic Blood Pressure; [b]Heart Rate; [c]Double Product; [d]Filtered Air; [e]Particles + O3; [f]Particles alone.

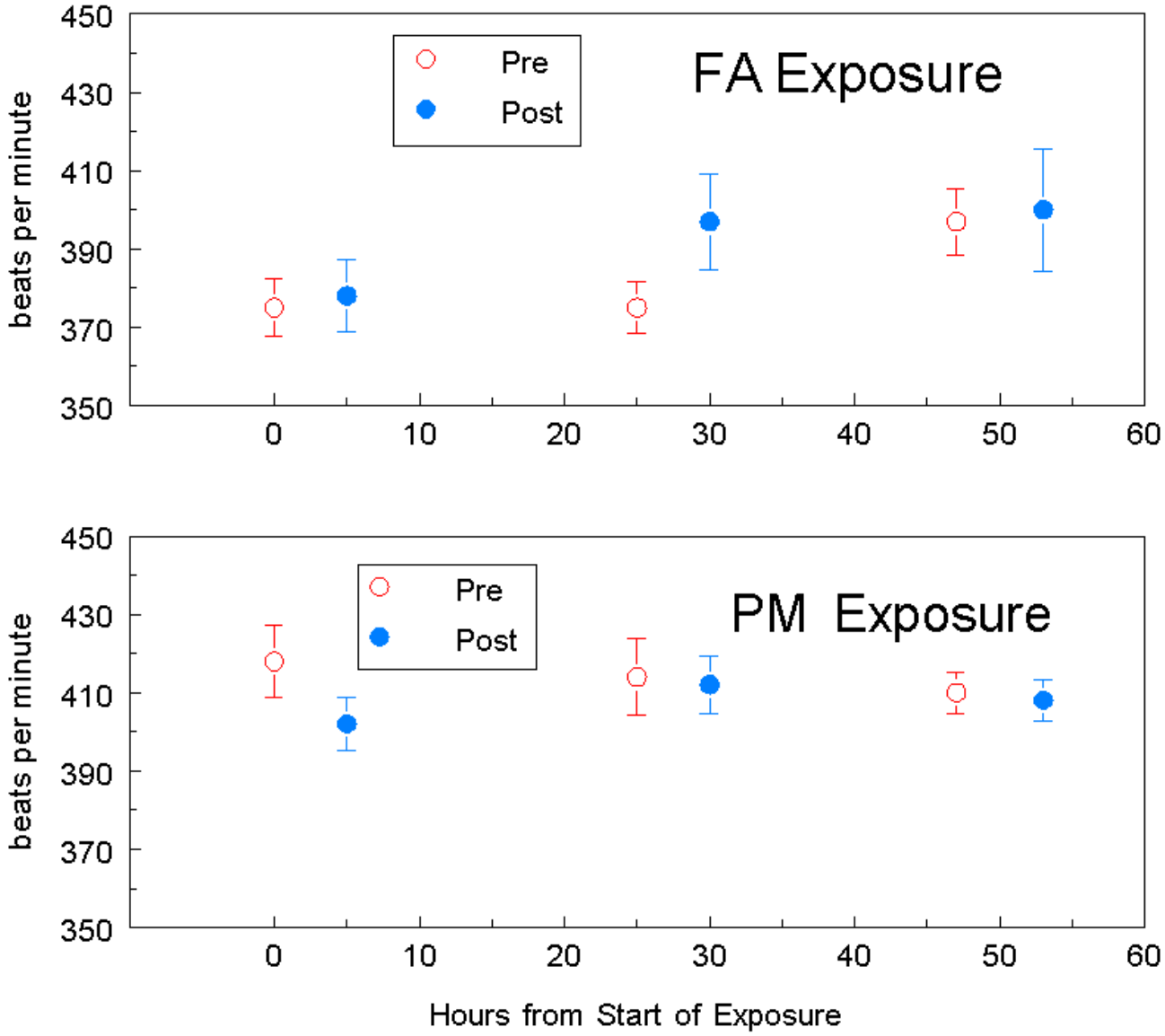
Single Day vs. Multiple Day Exposures

- Blood Pressure
- Heart Rate
- Rate x Pressure Product
- Heart rate Variability

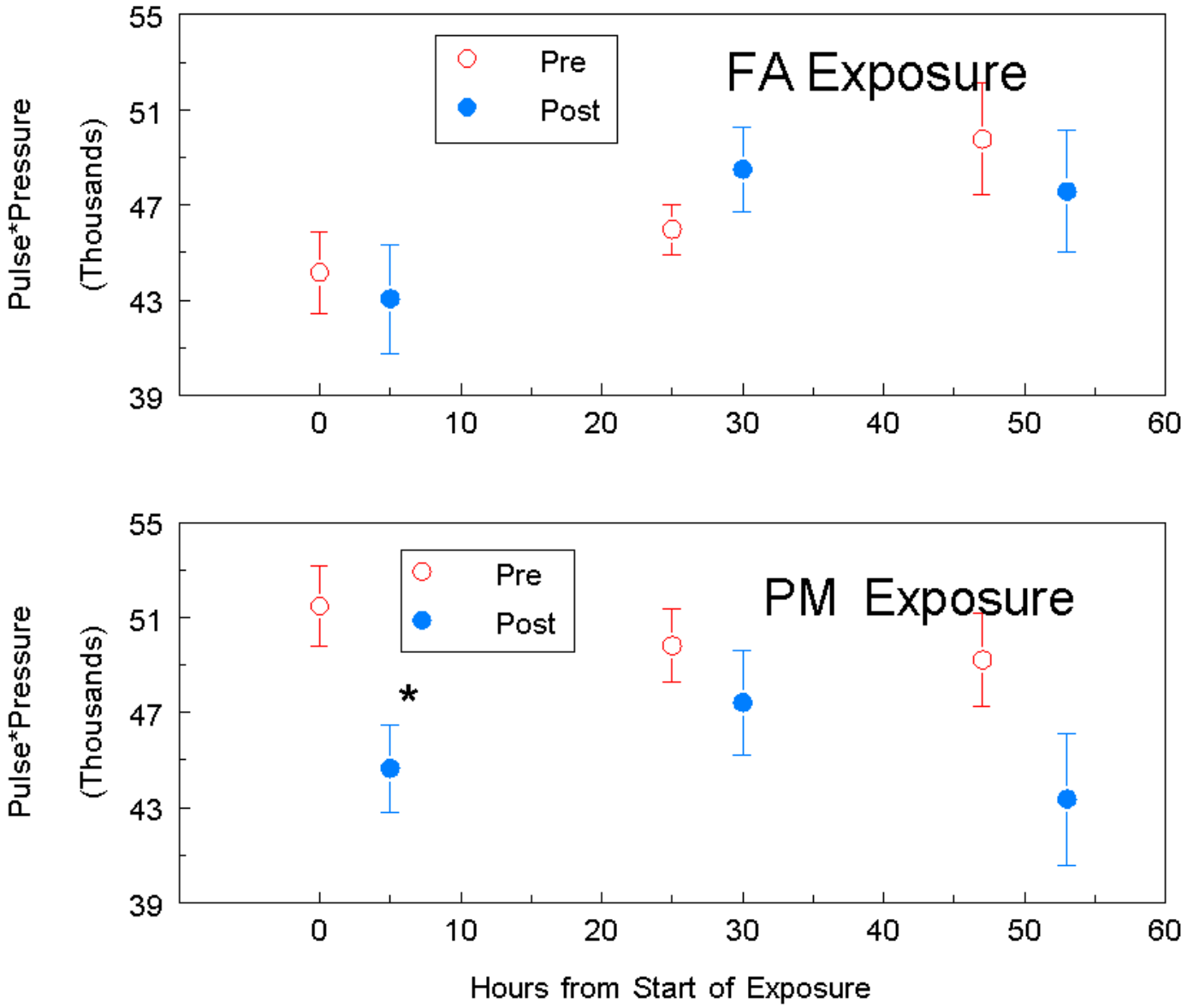
Blood Pressure was Moderately Reduced After PM Exposures but did not show Progressive Changes



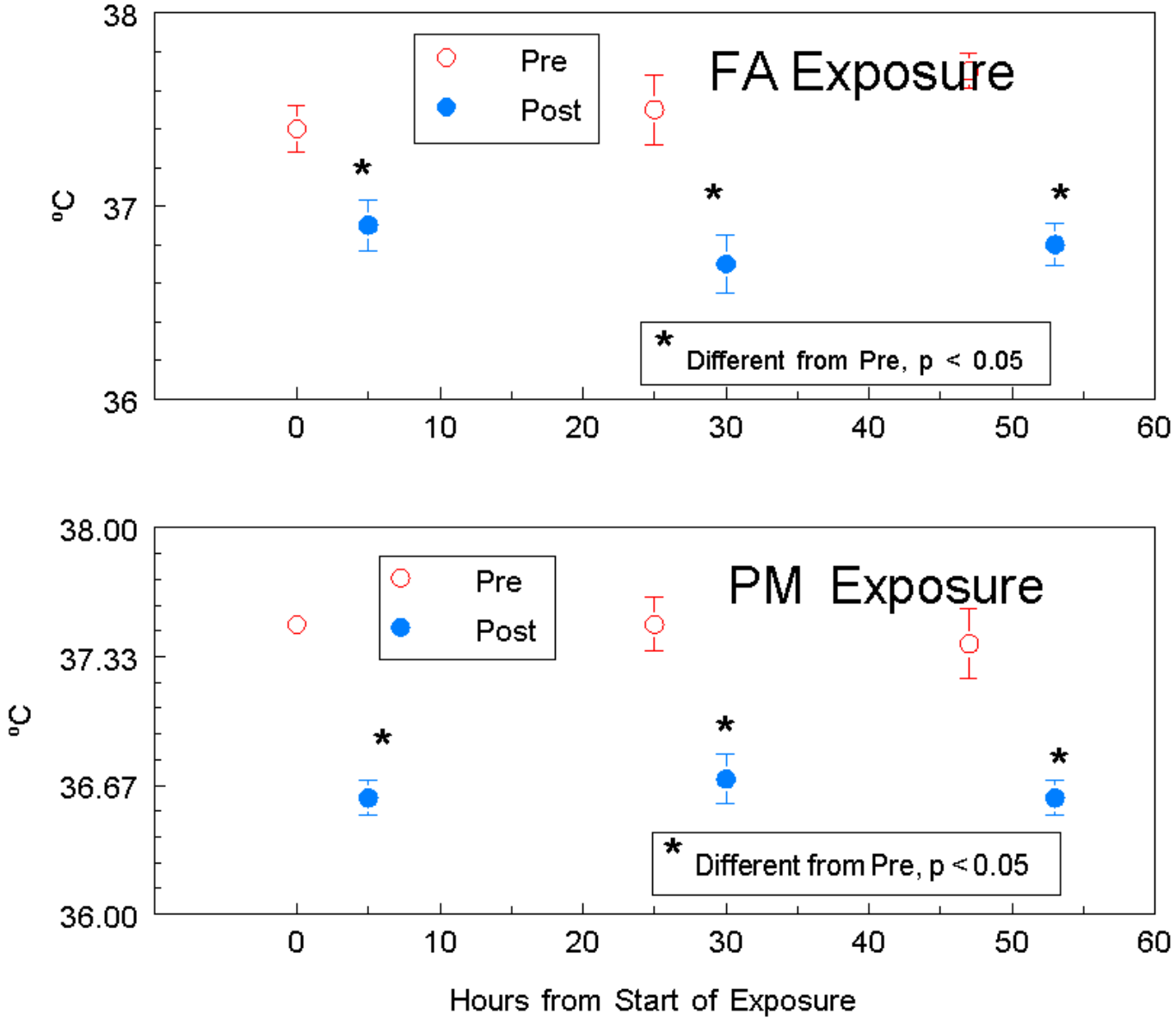
Heart Rate did not Show Significant Changes



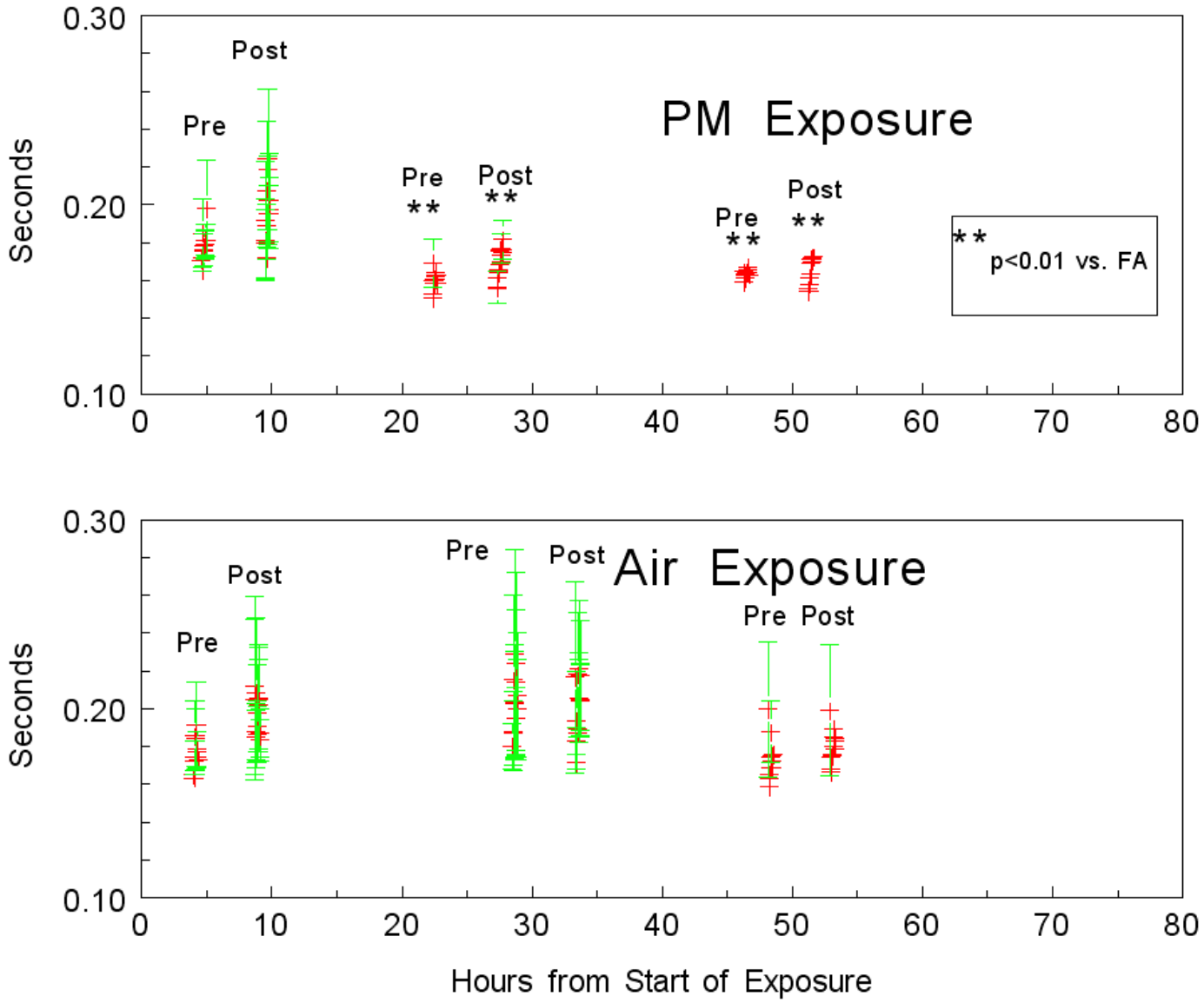
Double Product Was Reduced After PM Exposures but not After FA Exposures



Stress-induced changes in body temperature were examined for possible confounding effects



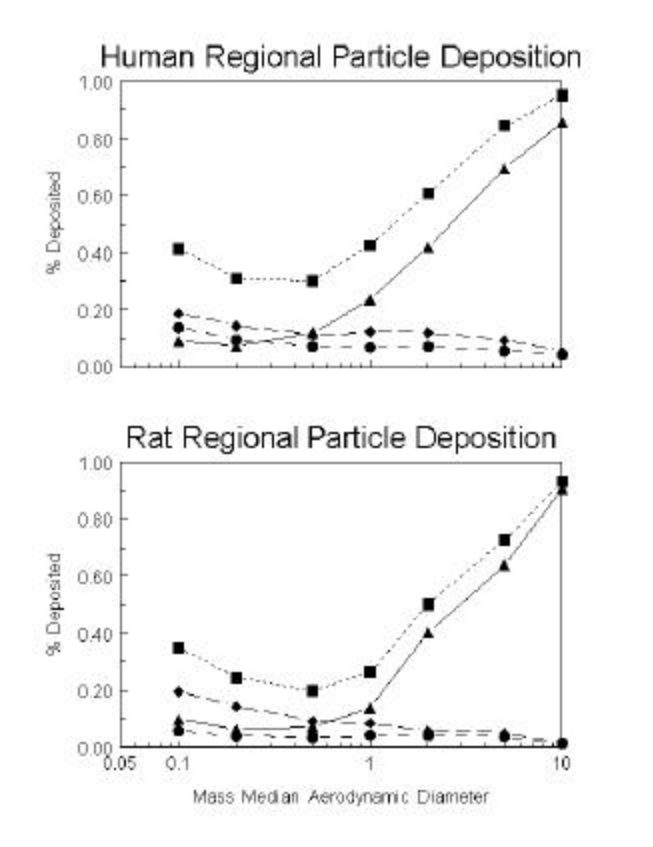
Heart Rate Variability Showed Cumulative Effects of 3-Day Exposures



Comparison to Human Study

- Both human and animal studies demonstrated PM-induced changes in HRV for the same exposure.
- Are doses comparable?

Dose Comparison



Estimated deposition in rat and human lungs as a function of Mass Median Aerodynamic Diameter (=Total Deposition, = Upper Respiratory Tract Deposition [non-thoracic], = Tracheobroncheal Deposition,?=Gas Exchange Region Deposition [Alveolar]).

Approximately 5% of inhaled particles with an MMAD of 0.7 µm would deposit in the gas exchange region of the rat's lung while approximately 8% would deposit in the comparable region of the human lung.